Neurobiology of Learning and Memory xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Paradoxical sleep: A vigilance state to gate long-term brain plasticity?

Pascal Ravassard [1], Al Mahdy Hamieh [2], Gaël Malleret, Paul-Antoine Salin *

Centre National de la Recherche Scientifique (CNRS), Unité Mixte de Recherche 5292, Institut National de la Santé et de la Recherche Médicale (INSERM), Unité 1028, France
Physiopathology of the Sleep Neuronal Networks, Lyon Neuroscience Research Center, F-69008 Lyon, France
University Lyon 1, F-69000 Lyon, France

ARTICLE INFO

Article history:
Received 1 August 2014
Revised 11 November 2014
Accepted 19 November 2014
Available online xxxx

Keywords:
Zif268 also known as EGR-1
c-Fos
BDNF
LTP and L-LTP
Post-learning paradoxical sleep window

ABSTRACT

Memory consolidation is the process for long-term storage of information and protection against interferences. It has been proposed that long-term potentiation (LTP), the long-lasting enhancement of synaptic transmission, is a cellular model for memory consolidation. Since consolidation of several forms of memory is facilitated by paradoxical sleep (PS) we ask whether PS modulates the cellular and molecular pathways underlying LTP. The long-lasting form of LTP (L-LTP) is dependent on the activation of transcription factors, enzymatic cascades and the secreted neurotrophin BDNF. By using PS deprivation, immunohistochemistry and quantitative real-time polymerase chain reaction (qPCR), we showed that an increase in PS amount (produced by rebound in PS deprived rats) is able to up-regulate the expression level of transcription factors Zif268 and c-Fos as well as Arc and BDNF in the CA1 and CA3 areas of the hippocampus. Several studies involved these factors in dendritic protein synthesis and in long-term structural changes of synapses underlying L-LTP. The present study together with the work of others (Ribeiro et al., 2002) suggest that by this mechanism, a post-learning increase in PS quantity (post-learning PS window) could convert a transient form of LTP to L-LTP.

1. Introduction

Since its discovery by Dement and Kleitman (1957) and Jouvet, Michel, and Courjon (1959) more than fifty years ago, paradoxical sleep (PS, or REMS for rapid-eye-movement sleep) is an important field of medical and neuroscience research. Several aspects concerning PS, in particular its precise role in cognitive functions are currently eliciting tremendous investigation. Recent studies in human have clearly identified a role for PS in procedural and emotional memory as well as in emotional processing (Rasch & Born, 2013; Walker, 2009). Functional brain imaging studies have also revealed that several cortical areas involved in memory processing are activated by PS such as the amygdaloid complexes and the anterior cingulate cortex (Maquet et al., 1996). In rodents, an overwhelming number of studies also suggest a role for PS in spatial and emotional memory (Hennevin, Hars, Maho, & Bloch, 1995; Smith, 1996). However, only few studies examined the mechanisms by which PS may facilitate these forms of learning and memory.

It has been well established that long-term potentiation (LTP), a major form of long-term synaptic plasticity, is a cellular mechanism required for learning and memory consolidation (Malenka & Bear, 2004; Wang & Morris, 2010; Whitlock, Heynen, Shuler, & Bear, 2006). Several decades of electrophysiological and biochemical studies have shown that LTP relies on a small number of key molecular targets such as the glutamate α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) and N-methyl-D-aspartate receptor (NMDAR) and enzymatic cascades located in dendritic spines of pyramidal cells (Malenka & Bear, 2004). LTP is now commonly divided in two forms, a transient one that rapidly fades over few hours and a late form that can be sustained over several hours, days and even weeks (Govindarajan, Kelleher, & Tonegawa, 2006). This late form of LTP (L-LTP), potentially responsible for synaptic consolidation, requires repeated synaptic activation. During the synaptic consolidation period, which has been hypothesized to precede system consolidation (Frankland & Bontempi, 2005), memory traces could be stored by the reinforcement of existing synaptic connections and the

* Corresponding author at: CNRS UMR 5292 – Inserm U 1028, Centre de Recherche en Neurosciences Lyon (CRNL), Université Lyon 1, Faculté de Médecine Laënnec, 7-11 rue G. Paradin, 69372 Lyon Cedex 08, France.
E-mail address: paul.salin@sommeil.univ-lyon1.fr (P.-A. Salin).
[1] Present address: University of California, Los Angeles, Department of Physics & Astronomy, Department of Neurology, 475 Portola Plaza, Room 5–170 Knudsen Hall, Los Angeles, CA 90095, United States.
[2] Present address: INSERM U1107, UdA Neuro-Dol Equipe Pharmacologie Fondamentale et Clinique de la Douleur, Facultés de médecine et de pharmacie, 28 place Henri-Dunant, BP38, 63001 Clermont-Ferrand, France.

http://dx.doi.org/10.1016/j.nlm.2014.11.013
1074-7427/

growth of new synaptic connections (Yang et al., 2014). In contrast with transient LTP, L-LTP involves *de novo* protein synthesis induced by dendritic translation or nuclear transcription under the control of transcription factors. This synthesis of new proteins is under the control of neuronal enzymatic cascades relying on mitogen-activated protein kinases (MAPK) and cAMP-dependent protein kinase (protein kinase A, PKA).

Interestingly, it has been shown that several enzymatic cascades involved in long-term synaptic plasticity are regulated by sleep states (Abel, Havekes, Saletin, & Walker, 2013; Tononi & Cirelli, 2014). It is known that sleep states modulate synaptic transmission and plasticity in the hippocampus and neocortex (Bramham & Srebro, 1989; Winson & Abzug, 1977). It has been proposed that slow wave sleep (SWS) could contribute to a global synaptic downscaling after wakefulness (Tononi & Cirelli, 2006). In agreement with this Synaptic Homeostasis Hypothesis (SHY), the response to evoked frontal Local Field Potentials (LFP) and AMPAR subtype GluA1 expression are down regulated during sleep (Vyazovskiy, Cirelli, Pfister-Genskow, Faraguna, & Tononi, 2008). Moreover, calcineurin, a protein phosphatase involved in long-term synaptic depression (LTD), is upregulated during sleep while the immediate early gene (IEG) activity-regulated cytoskeleton-associated protein (*Arc* also known as Arg3.1) and the trophic factor Brain-derived neurotrophic factor (BDNF) are upregulated by wakefulness (Cirelli, Gutierrez, & Tononi, 2004). In this model, the role of PS is unclear, although a recent study suggests that it may contribute to a homeostasis of hippocampal excitability during SWS (Grosmark, Mizuseki, Pastalkova, Diba, & Buzsaki, 2012). Alternatively, several studies suggest that sleep might play an active role and in particular may facilitate long-term synaptic plasticity (Rasch & Born, 2013). This later model suggests that hippocampal sharp-wave ripples (SWR) are associated with hippocampal and neocortical replays which reinforce/induce synaptic potentiation (Replay-Transfer-Potentiation, RTP). SWR-induced replays during SWS may facilitate LTP induction and then PS may convert it into L-LTP. According to this model, experiments using the IEG *Zif268* (also known as Egr1, Krox-24, NGF1-A, Zenk), a transcription factor required for L-LTP (Jones et al., 2001), suggest that waves of *Zif268* up-regulation propagate during PS from the hippocampus to the neocortex after hippocampal LTP (Ribeiro et al., 2002).

We have shown that the expression level of some AMPAR and NMDAR subtypes is down-regulated in the CA1 area of the dorsal hippocampus by PS deprivation and that this decrease is associated with a reduction of MAPK activity in the same area (Ravassard et al., 2009). Conversely, sleep deprivation impaired L-LTP in the dorsal CA1 area. Therefore, these studies suggest that sleep states, and PS in particular, play an important role in regulating L-LTP and its key molecular targets. However, some questions remain unanswered. Since L-LTP in CA1 pyramidal cells seem to be required for several forms of long-term memory, are these cells activated by sleep states? BDNF, a secreted protein that plays an important role in brain development, is present in adult animals in CA1–CA3 pyramidal cells and is required for converting LTP in L-LTP (Bramham & Messaoudi, 2005; Govindarajan et al., 2006). Is BDNF expression modulated by PS?

To answer these questions, we first used immunohistochemistry and quantitative PCR of the IEG *Zif268*, *c-Fos* and *Arc* acting as indirect markers of neuronal activity and synaptic plasticity (Bramham, Worley, Moore, & Guzowski, 2008; Cirelli & Tononi, 2000; Fleischmann et al., 2003; Veyrac, Besnard, Caboche, Davis, & Laroche, 2014). Second, we examined the expression of BDNF in the same hippocampal areas. In these experiments, PS amount was modulated by using a PS deprivation method. After PS deprivation, the animals were subjected to a recovery of their PS debt which induced a selective and sustained increase in PS amount (PS rebound, PSR). Our results suggest that increasing PS by PS rebound up-regulates markers of synaptic plasticity and *BDNF* in the CA1–CA3 areas of the hippocampus.

2. Materials and methods

2.1. Polygraphic recording of vigilance states

All procedures were approved by the institutional animal care and use committee of the University of Lyon 1 (protocol BH2006-09) and were conducted in accordance with the French and European Community guidelines for the use of research animals. Experiments were performed on male Sprague Dawley rats (7–8 weeks old, 250–350 g, Charles River Laboratories, France). Animals were implanted under chloral hydrate anesthesia (400 mg/kg) to monitor electroencephalogram (frontal and parietal EEG) and electromyogram (nuchal EMG). EEG and EMG signals were amplified (X500), filtered (0.5–100 Hz), digitized at 250 Hz, and collected with CED using Spike-2 interface software (Cambridge Electronic Design). Hypnograms were scored off-line based on EEG and EMG signals by using a 10-s analysis window. The episodes of each vigilance state were identified according to the following criteria: waking (W) was characterized by desynchronized low-voltage and high-frequency activity EEG and by sustained neck muscle

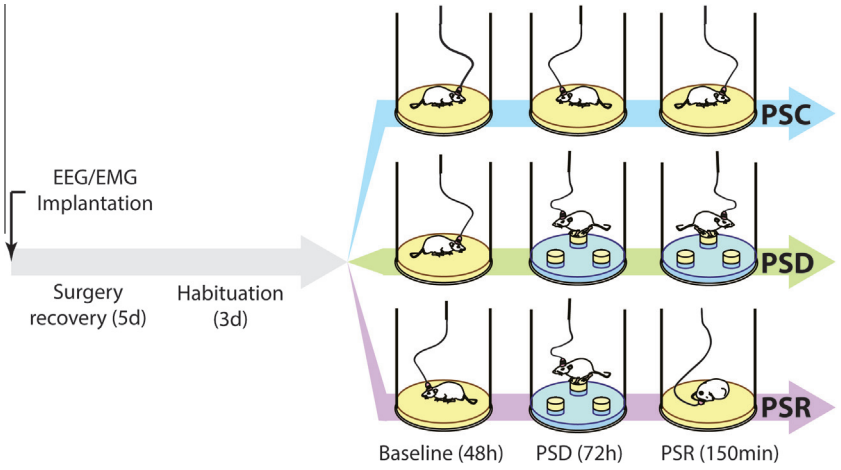

Fig. 1. Experimental paradigm. Three group of rats were studied: control (PSC), PS deprived (PSD) during 75 h and PS rebound for 2h30 after 72 h of PS deprivation (PSR). Multiple platforms were added to prevent the stress induced in rats by immobilization.

Please cite this article in press as: Ravassard, P., et al. Paradoxical sleep: A vigilance state to gate long-term brain plasticity? *Neurobiology of Learning and Memory* (2014), http://dx.doi.org/10.1016/j.nlm.2014.11.013

tone at the EMG; SWS was characterized by high-voltage slow waves (1.5–4 Hz) and spindles (10–14 Hz) combined with low muscle tone; PS episodes were identified by a decrease in the EEG amplitude and a prominent theta rhythm (5–9 Hz) associated with muscle atonia at the EMG.

2.2. Paradoxical sleep deprivation

Thirty-four animals spent 3–5 days (d) in recovery from surgery in individual standard Plexiglas containers under standard laboratory conditions (i.e., 12/12-h light–dark cycle with light-on at 07:00, 22–24 °C ambient temperature, food and water *ad libitum*). After a 5-d habituation period to the recording environment, the protocol was performed over a 6-d period during which most of the rats were recorded. Baseline sleep was recorded during 48 h for the three experimental groups of rats used in this study: PS control (PSC), PS deprivation (PSD) and PS rebound (PSR, Fig. 1). PSD rats underwent a 72-h PS deprivation on multiple platforms (MP) in a standard container filled with water (2–3 cm depth). Three platforms (6.2 cm diameter, 7–12 cm height) were spaced 7–8 cm apart, so that the rat could easily move between them. This constitutes a modification of the classic "flower pot" technique that resulted in a selective PSD (McDermott et al., 2003; Mendelson, Guthrie, Frederick, & Wyatt, 1974). The PS deprivation is obtained when a rat is placed on top of an upside down flowerpot which is located in a bucket of water. This method is designed to prevent the generation of PS episodes while allowing slow wave sleep. MP were used to prevent the stress induced in rats by immobilization on a single platform (McDermott et al., 2003; Ravassard et al., 2009). This was confirmed by measuring blood corticosterone level in the three groups of rats. At the time of sacrifice (after 72 h), plasma corticosterone level was not different between PSC and PSD groups (PSC: 53.6 ± 13.7 ng/ml, $n = 11$; PSD: 85.4 ± 23 ng/ml, $n = 9$, $p > 0.05$). However, corticosterone level was different between PSR animals and the other groups (PSR: 19.9 ± 8 ng/ml, $n = 10$; $p < 0.05$ with PSC and PSD). During PSD, food and water were available *ad libitum*, and the container was cleaned daily. During this cleaning period, the rats were placed in a dry cage for 30–45 min where they stayed awake (grooming and exploratory behavior) as evidenced by direct examination EEG/EMG activity and video. PSR rats underwent the same deprivation protocol as PSD rats and were maintained on MP until the end of PSD, at which point the rats were placed in a standard container at 07:00 on day 6. After ≈40 min of grooming and exploration, the recovery of the PS debt induced a sustained and selective increase in PS amount (PS rebound) during several hours. PSR rats were euthanized 150 min after the first PS episode (i.e., approximately at 10:00, day 6). Basal sleep was assessed with the PS control (PSC) group, which remained in a standard container during the protocol. Statistical significance was quantified by analysis of variance (ANOVA). In case of significance ($p < 0.05$), the Fisher test was followed by a *post hoc* test to identify significant pairwise differences with a non-parametric paired sample Wilcoxon signed rank test.

2.3. Immunohistochemistry

Seventeen rats (6 PSC, 5 PSD and 6 PSR) were perfused with Ringer solution containing 0.1% heparin followed by a fixative solution (4% paraformaldehyde in 0.1 M phosphate buffer). Coronal sections containing hippocampi (25 μm thick) obtained with a cryostat were then incubated with Zif268 (1:2000, Santa Cruz) rabbit polyclonal antibody for 72 h at 4 °C with constant rotation. After 2-h incubation in biotinylated anti-rabbit secondary IgG (1:1000, Rockland, Tebu-bio), sections were processed with avidin–biotin horseradish peroxidase complex (ABC, Elite Kit from Vector Laboratories), and the reaction was visualized with diaminobenzidine (DAB, Sigma). Zif268-positive cell density was quantified on 3–5 sections of the left and right hippocampus by using a microscope equipped with a digital camera and an image analyzing system (Mercator, Explora Nova). One-way ANOVA and unpaired 2-tailed Student *t*-test were performed to compare the differences between groups.

2.4. Quantitative real-time polymerase chain reaction (qPCR)

2.4.1. Tissue dissection

Slices (1-mm thick) were rapidly dissected on ice from the dorsal and ventral hippocampus of seventeen rats containing mainly CA1–CA3 areas after cutting at the hippocampal fissure. Hippocampal tissues were directly introduced in dry aliquots and immersed into liquid N_2 and stored at −80 °C until use.

Total RNA was extracted from the hippocampus using the RNeasy Lipid Tissue mini kit (Qiagen) according to the manufacturer's protocol. qPCR was performed with a Light Cycler Faststart DNA master SYBR Green1 kit (Roche, at Neurobiotech service, Lyon). RNA samples were treated with RNase-free DNase Set (Qiagen) to eliminate DNA contamination. Total RNA quality and yield were assessed using a bioanalyzer (Agilent2100, Agilent Technologies). Primer pairs were generated to amplify specific 100–200 bp fragments from rat (Genebank) for *Arc* (Forward, 5′ to 3′: TCCTGCAGATTGGTAAGTGC), *c-Fos* (TTGTAGTGACACCTGAGAGC), *Zif-268* (ACAGTGGCAACATTT GTGG), and *BDNF* (GTGACAGTATTAGCGAGTGG). Expression levels were normalized with the following housekeeping genes: beta-actin (GCATTGCTGACAGGATGCAG), HPRT (Hypoxanthine guanine phosphoribosyl transferase: CAGGCCAGACTTTGTTGGAT) and YWHAE (Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, GTTTGCGGTAATGGTTTTGC), which were used as internal controls for quantitative analysis. Similar results were obtained with these three housekeeping genes. The Tukey–Kramer multiple comparison test was performed to compare level of expression between groups.

3. Results

First, we sought to identify what neuronal population was activated by PS rebound in the hippocampus. We carried out an immunohistochemical study on three experimental groups of rats undergoing large manipulations of PS amount by comparing control conditions (PSC), PS deprivation (PSD) and PS rebound (PSR) induced by the restoration of PS debt following PS deprivation, to determine whether PS amount was able to modulate Zif268 expression. Using this protocol we previously found (Ravassard et al., 2009) that during the 150 min period (corresponding to the total duration of PS rebound for comparison) before sacrifice, PS quantity was drastically decreased in PSD compared to PSC ($p < 0.001$) and increased by nearly threefold in PSR ($p < 0.01$, Fig. 2). During the same period, SWS amount was not significantly different between PSD and PSR ($p > 0.05$) but decreased as compared with PSC ($p < 0.05$ for PSD and 0.01 for PSR). As illustrated in Fig. 3, PS rebound induced a twofold increase in the density of Zif268 positive neurons in the CA1 area of the dorsal hippocampus. This increase in density of Zif268 positive cells after PS rebound was much less in the CA3 area and dentate gyrus of the hippocampus. This increase in density of Zif268 positive cells was limited to two neuronal types, the pyramidal cells in CA1 and CA3 areas and the granular cells in the dentate gyrus. In the dorsal CA1, we also observed a slight but non-significant increase in density of Zif268 positive cells during PSD compared to PSC ($p > 0.05$).

Next, we asked whether the increase in density of Zif268 positive cells was homogenous in all the hippocampus. Several anatomical

Please cite this article in press as: Ravassard, P., et al. Paradoxical sleep: A vigilance state to gate long-term brain plasticity? *Neurobiology of Learning and Memory* (2014), http://dx.doi.org/10.1016/j.nlm.2014.11.013

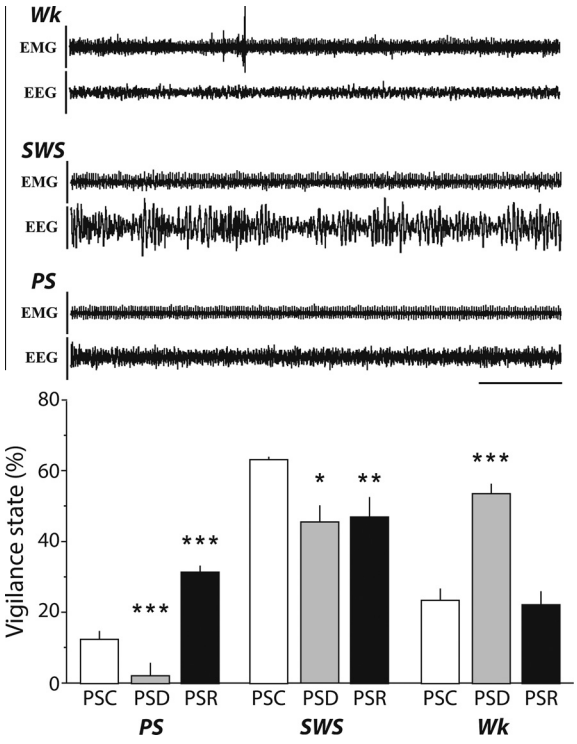

Fig. 2. Sample EEG and EMG recordings showing characteristic episodes of wakefulness (Wk), slow-wave sleep (SWS) and paradoxical or REM sleep (PS). Scale: 2 mV, 5 s. Vigilance states quantification showing the percentage of time spent in each state during the last 150 min of the protocol (PSC $n = 6$, PSD $n = 9$, PSR $n = 10$).

tracing, behavioral and electrophysiological studies have suggested that the dorsal and ventral hippocampus process respectively spatial information and emotions (Fanselow and Dong, 2010). As shown in Fig. 3, an increase in the density of Zif268 positive neurons was also observed in the ventral hippocampus after PS rebound. In ventral CA1, there was no increase in the density of Zif268 positive cells of PSD rats compared to PSC rats.

In order to quantify the expression level of *Zif268*, we carried out quantitative real-time polymerase chain reaction (qPCR) in the CA1–CA3 area of the dorsal hippocampus (see Methods). Our results indicate that there was more than a twofold increase in the expression level of *Zif268* in the PSR group compared to PSC (Fig. 4). This increase of *Zif268* expression level was even more pronounced in the CA1–CA3 area of the ventral hippocampus. We then asked whether other indirect markers of neuronal activity and synaptic plasticity were up-regulated during PS rebound. We observed a weak but significant increase in the IEG *c-Fos* in the CA1–CA3 region of dorsal hippocampus. However, we found more than a fourfold increase in the expression of this IEG in the CA1-CA3 area of the ventral hippocampus. Finally, we also examined the modulation of *Arc* expression level by PS amount. The expression level of Arc was also significantly increased the CA1–CA3 area of the dorsal and ventral hippocampus.

To further understand the effect of PS amount on mechanisms of long-term synaptic plasticity, we examined the expression level of *BDNF*, a key actor of long-term synaptic plasticity and dendritic protein synthesis. Our qPCR analysis clearly showed that *BDNF* expression level increases more than twice in the CA1–CA3 area of the dorsal and ventral hippocampus in the PSR group compared to PSC (Fig. 4). Several genes playing key roles in long-term synaptic plasticity were thus up-regulated in the CA1–CA3 area of the hippocampus by an increase in PS amount. Altogether, these results suggest that PS may play an important role in the synaptic consolidation processes.

4. Discussion

Altogether, our results suggest that the increase in PS amount following PS deprivation selectively up-regulates known biomarkers of long-term synaptic plasticity in the CA1–CA3 areas of the hippocampus. Fig. 5 summarizes the results that we obtained in the present study and previously in Ravassard et al., 2009. We found that both synaptic transmission and plasticity (LTP) were impaired in dorsal CA1 after PSD. The protein expression of their key effectors such as GluR1 and NR1, as well as the MAPK cascade and its main messenger the phosphorylated form of extracellular

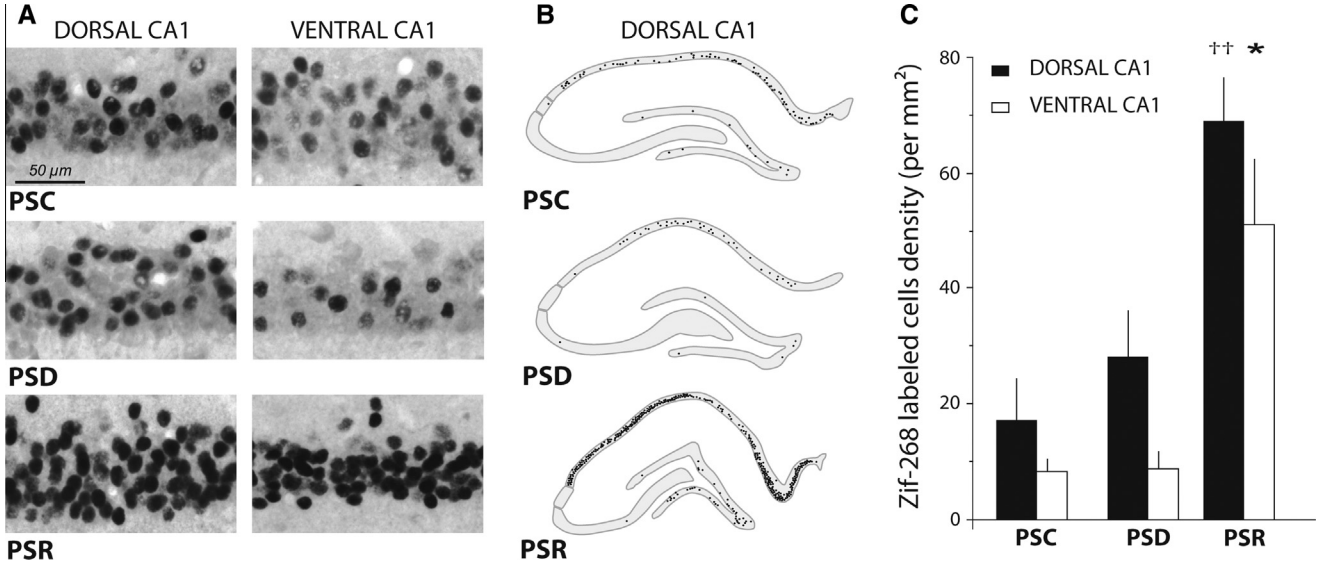

Fig. 3. Increase in the density of Zif268-positive neurons in the hippocampus after PS rebound. A. Microphotographs of Zif268-positive cells in the CA1 area of the dorsal and ventral hippocampus in the three groups of rats: PSC, PSD and PSR. B. Distribution of Zif268-positive cells in coronal sections of the dorsal hippocampus of the three groups. C. Quantification of the density of Zif268-positive cells in 6 PSC, 5 PSD and 6 PSR rats. A significant increase in the density of Zif268 positive cells was observed in the PSR group compared to PSC and PSD groups in the CA1 area of the dorsal hippocampus ($^{††}p < 0.004$, $^{*}p < 0.03$).

Please cite this article in press as: Ravassard, P., et al. Paradoxical sleep: A vigilance state to gate long-term brain plasticity? *Neurobiology of Learning and Memory* (2014), http://dx.doi.org/10.1016/j.nlm.2014.11.013

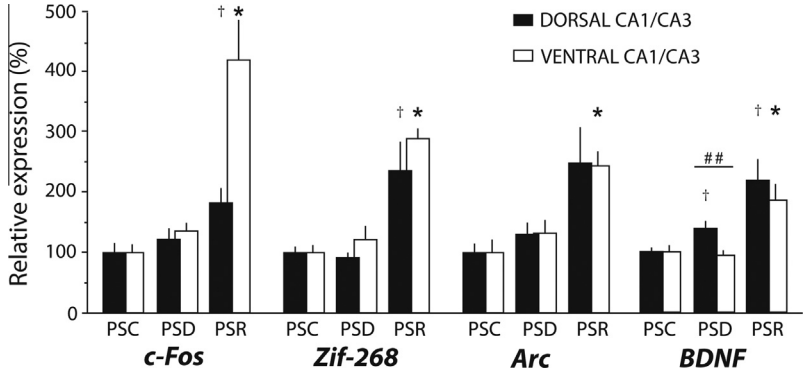

Fig. 4. Real-time PCR analysis of *Zif268*, *c-Fos*, *Arc* and *BDNF* expression level in the hippocampus after paradoxical sleep rebound. Dorsal CA1–CA3, PSR vs PSC: *c-fos*: $p < 0.03$, *zif-268:* $p < 0.03$, *Arc*: $p < 0.05$, BDNF: $p < 0.01$; Ventral CA1–CA3, PSR vs PSC: *c-fos*: $p < 0.01$, *zif-268*: $p < 0.01$, *Arc:* $p < 0.01$, *BDNF:* $p < 0.01$ ($n = 17$). The expression level of these genes were normalized with the housekeeping gene *β-actin.*

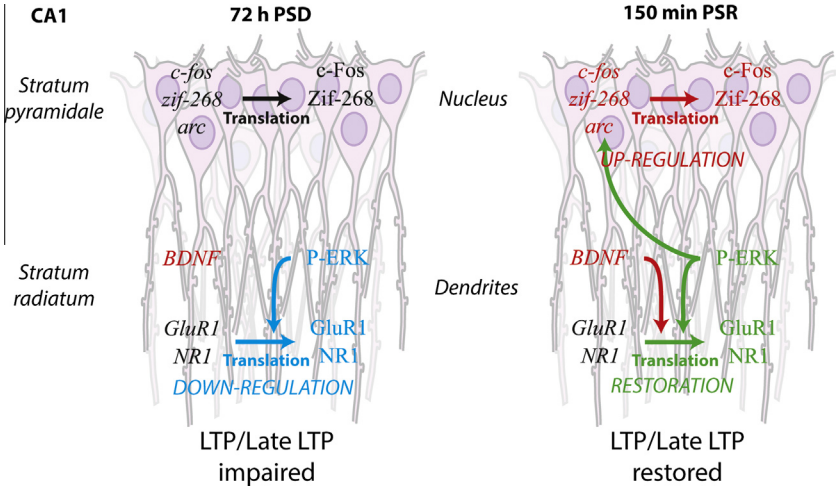

Fig. 5. Summary of the results obtained after paradoxical sleep rebound for CA1 pyramidal cells of the hippocampus. Paradoxical sleep deprivation down-regulates the expression level of ERK-MAPK cascade (ERK phosphorylation), the AMPAR subtype GluR1 and the NMDAR subtype NR1(arrows in green). In contrast paradoxical sleep rebound up-regulates BDNF, and the transcription factors Zif268, c-Fos and Arc (arrows in red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

signal-regulated kinase (p-ERK), were down-regulated (in blue). Both LTP and these protein effectors were restored (in green) to control levels after rebound (Ravassard et al., 2009). The present study revealed an up-regulation of several IEGs (c-Fos, Arc, Zif268, BDNF) mRNA and protein expression (in red) after 150 min of PS rebound, while their levels were normal after 72 h PSD. Therefore, PS amount has a deep impact on L-LTP in the hippocampus by regulating several molecular targets in pyramidal cells: a decrease in PS amount down-regulates the MAPK cascade and glutamate receptors, while PS recovery restores their levels and up-regulates several transcription factors. We hypothesize that during PSD, the impairment of the MAPK pathway leads to a reduction of glutamate receptors and a lack of synaptic plasticity. During PS recovery, this pathway is restored and via dendritic translations, reconstitutes the glutamate receptors pool and restores LTP. This local wave of translation is completed by an overall wave that activates nuclear translation of several transcription factors for a slower reconstitution of key molecular targets maintaining plasticity. Our work does not capture the whole kinetic of these two waves of activation, as the dendritic wave of regulation seems to be achieved after 150 min of PS rebound. Pharmacological and selective manipulations of local and global translation should be used to test these proposed mechanisms during PS recovery, and ultimately during natural sleep states. Another question is to know whether these local regulatory mechanisms are triggered endogenously or exogenously. One can look at PS as a global state in the hippocampal network either facilitating or eliciting these regulations. Two potential factors could be involved the prominent theta oscillations and the major acetylcholine tone during PS that are known to induce long-term plasticity in the hippocampus (Greenstein, Pavlides, & Winson, 1988; Hasselmo, 2006; Holscher, Anwyl, & Rowan, 1997; Marrosu et al., 1995).

It has been suggested for a long time that PS plays an important role in learning and memory consolidation (Maquet, 2001; Smith, 1995). Most compelling evidences arise from studies on emotional memory and visual skill learning in humans (Karni, Tanne, Rubenstein, Askenasy, & Sagi, 1994; Nishida, Pearsall, Buckner, & Walker, 2009; Walker, 2009) Rasch & Born, 2013). However, the molecular mechanisms underlying such facilitating effect of PS on these cognitive processes are still largely unknown. To tackle the molecular role of PS, we focused here on the effect of PS rebound, a major homeostatic property of sleep in mammals. In basal conditions, PS episodes are short compared to waking and SWS episodes in rodents. Therefore, we increased PS duration by PS rebound after PS deprivation in order to compensate for the low amount of PS observed in basal conditions. It has been shown in rodents that numerous types of learning induce a selective increase in PS amount (Hennevin et al., 1995; Smith, 1995). For

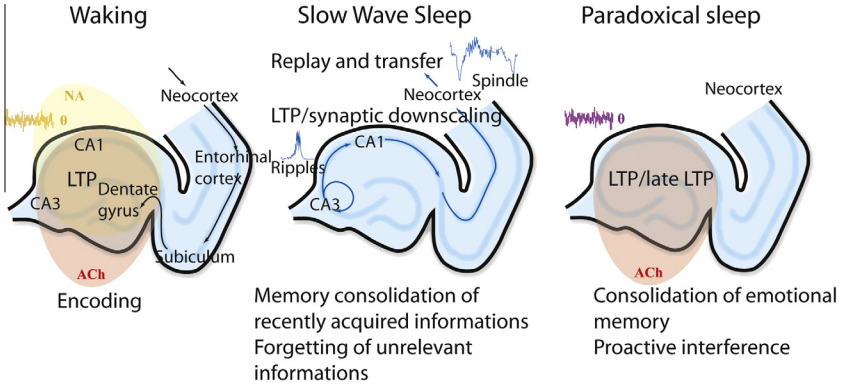

Fig. 6. Model proposed for the paradoxical sleep role in memory consolidation. Recent results suggest that paradoxical sleep may induce synaptic consolidation after neuronal replay during slow wave sleep in the hippocampus and interconnected circuits.

instance, spatial learning tasks and emotional conditioning produce an increase in PS quantity after a delay (ie post-learning PS window). It has been shown that this post-learning PS window is required for forms of spatial tasks dependent on the hippocampus (Datta, Mavanji, Ulloor, & Patterson, 2004; Smith, 1995). The selective alteration of PS during this window impairs emotional memory (Datta et al., 2004) Ravassard et al., unpublished results). Therefore, we think that the present paper adds substantial data to our understanding of the underlying role of PS in memory consolidation. We hypothesize that the up-regulation in BDNF expression level as well as in the IEG Zif268, c-Fos and Arc could be induced by an increase in PS amount during post-learning period and could trigger a molecular switch from LTP to L-LTP in the CA1–CA3 areas of the hippocampus. Post-learning PS window could thus be a transient period to gate synaptic consolidation in areas involved in memory processes.

Our results should be discussed in link with the work of Ribeiro and Pavlides on *Zif268* expression during PS (Ribeiro, Goyal, Mello, & Pavlides, 1999; Ribeiro et al., 2002, 2007). This work suggests that new environment and hippocampal LTP induce an up-regulation of *Zif268* expression level in waking and PS which propagate from the hippocampus to other cortical areas during PS episodes. This propagation in different brain areas suggests that PS may facilitate synaptic consolidation in interconnected brain areas. These waves of *Zif268* activation may contribute to facilitate hippocampal-dependent forms of memory. It could be important to determine whether the up-regulation of *Zif268* in these cortical areas is associated with an increase in BDNF expression. As proposed in Fig. 6, these roles of PS in synaptic consolidation could cooperate with the functions of SWS to promote neuronal reactivation and transfer of information between areas related to the hippocampus. It is known that PS amount are higher in youth compared to adult. We hypothesized a potential role of PS-induced increase in IEG and BDNF in experience-dependent forms of plasticity during development in particular for sensory systems (Seibt et al., 2012).

Acknowledgments

P.R. and the project were supported by the Cluster11 Rhône-Alpes grant to P.A.S.; A.M.H was supported by an ARC2 (Région Rhône-Alpes) grant to G.M. We thank Nadine Gay, Catherine Rey and the Plateform ProfileXpert for help with the qPCR analysis.

References

Abel, T., Havekes, R., Saletin, J. M., & Walker, M. P. (2013). Sleep, plasticity and memory from molecules to whole-brain networks. *Current Biology, 23*, R774–R788.

Bramham, C. R., & Messaoudi, E. (2005). BDNF function in adult synaptic plasticity: The synaptic consolidation hypothesis. *Progress in Neurobiology, 76*, 99–125.

Bramham, C. R., & Srebro, B. (1989). Synaptic plasticity in the hippocampus is modulated by behavioral state. *Brain Research, 493*, 74–86.

Bramham, C. R., Worley, P. F., Moore, M. J., & Guzowski, J. F. (2008). The immediate early gene arc/arg3.1: Regulation, mechanisms, and function. *Journal of Neuroscience, 28*, 11760–11767.

Cirelli, C., Gutierrez, C. M., & Tononi, G. (2004). Extensive and divergent effects of sleep and wakefulness on brain gene expression. *Neuron, 41*, 35–43.

Cirelli, C., & Tononi, G. (2000). Differential expression of plasticity-related genes in waking and sleep and their regulation by the noradrenergic system. *Journal of Neuroscience, 20*, 9187–9194.

Datta, S., Mavanji, V., Ulloor, J., & Patterson, E. H. (2004). Activation of phasic pontine-wave generator prevents rapid eye movement sleep deprivation-induced learning impairment in the rat: A mechanism for sleep-dependent plasticity. *Journal of Neuroscience, 24*, 1416–1427.

Dement, W. C., & Kleitman, N. (1957). Cyclic variations of EEG during sleep and their relation to eye movement, body motility, and dreaming. *Electroencephalography and Clinical Neurophysiology, 9*, 673–690.

Fanselow, M. S., & Dong, H. W. (2010). Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron, 65*, 7–19.

Fleischmann, A., Hvalby, O., Jensen, V., Strekalova, T., Zacher, C., Layer, L. E., et al. (2003). Impaired long-term memory and NR2A-type NMDA receptor-dependent synaptic plasticity in mice lacking c-Fos in the CNS. *Journal of Neuroscience, 23*, 9116–9122.

Frankland, P. W., & Bontempi, B. (2005). The organization of recent and remote memories. *Nature Reviews Neuroscience, 6*, 119–130.

Govindarajan, A., Kelleher, R. J., & Tonegawa, S. (2006). A clustered plasticity model of long-term memory engrams. *Nature Reviews Neuroscience, 7*, 575–583.

Greenstein, Y. J., Pavlides, C., & Winson, J. (1988). Long-term potentiation in the dentate gyrus is preferentially induced at theta rhythm periodicity. *Brain Research, 438*, 331–334.

Grosmark, A. D., Mizuseki, K., Pastalkova, E., Diba, K., & Buzsaki, G. (2012). REM sleep reorganizes hippocampal excitability. *Neuron, 75*, 1001–1007.

Hasselmo, M. E. (2006). The role of acetylcholine in learning and memory. *Current Opinion in Neurobiology, 16*, 710–715.

Hennevin, E., Hars, B., Maho, C., & Bloch, V. (1995). Processing of learned information in paradoxical sleep: Relevance for memory. *Behavioural Brain Research, 69*, 125–135.

Holscher, C., Anwyl, R., & Rowan, M. J. (1997). Stimulation on the positive phase of hippocampal theta rhythm induces long-term potentiation that can Be depotentiated by stimulation on the negative phase in area CA1 in vivo. *Journal of Neuroscience, 17*, 6470–6477.

Jones, M. W., Errington, M. L., French, P. J., Fine, A., Bliss, T. V., Garel, S., et al. (2001). A requirement for the immediate early gene Zif268 in the expression of late LTP and long-term memories. *Nature Neuroscience, 4*, 289–296.

Jouvet, M., Michel, F., & Courjon, J. (1959). Sur un stade d'activité électrique cérébrale rapide au cours du sommeil physiologique. *Comptes Rendus des Séances et Mémoires de la Société de Biologie, 153*, 1024–1028.

Karni, A., Tanne, D., Rubenstein, B. S., Askenasy, J. J., & Sagi, D. (1994). Dependence on REM sleep of overnight improvement of a perceptual skill. *Science, 265*, 679–682.

Malenka, R. C., & Bear, M. F. (2004). LTP and LTD: An embarrassment of riches. *Neuron, 44*, 5–21.

Maquet, P. (2001). The role of sleep in learning and memory. *Science, 294*, 1048–1052.

Maquet, P., Peters, J., Aerts, J., Delfiore, G., Degueldre, C., Luxen, A., et al. (1996). Functional neuroanatomy of human rapid-eye-movement sleep and dreaming. *Nature, 383*, 163–166.

Marrosu, F., Portas, C., Mascia, M. S., Casu, M. A., Fa, M., Giagheddu, M., et al. (1995). Microdialysis measurement of cortical and hippocampal acetylcholine release during sleep-wake cycle in freely moving cats. *Brain Research, 671*, 329–332.

Please cite this article in press as: Ravassard, P., et al. Paradoxical sleep: A vigilance state to gate long-term brain plasticity? *Neurobiology of Learning and Memory* (2014), http://dx.doi.org/10.1016/j.nlm.2014.11.013

McDermott, C. M., LaHoste, G. J., Chen, C., Musto, A., Bazan, N. G., & Magee, J. C. (2003). Sleep deprivation causes behavioral, synaptic, and membrane excitability alterations in hippocampal neurons. *Journal of Neuroscience, 23*, 9687–9695.

Mendelson, W. B., Guthrie, R. D., Frederick, G., & Wyatt, R. J. (1974). The flower pot technique of rapid eye movement (REM) sleep deprivation. *Pharmacology, Biochemistry and Behavior, 2*, 553–556.

Nishida, M., Pearsall, J., Buckner, R. L., & Walker, M. P. (2009). REM sleep, prefrontal theta, and the consolidation of human emotional memory. *Cerebral Cortex, 19*, 1158–1166.

Rasch, B., & Born, J. (2013). About sleep's role in memory. *Physiological Reviews, 93*, 681–766.

Ravassard, P., Pachoud, B., Comte, J. C., Mejia-Perez, C., Scote-Blachon, C., Gay, N., et al. (2009). Paradoxical (REM) sleep deprivation causes a large and rapidly reversible decrease in long-term potentiation, synaptic transmission, glutamate receptor protein levels, and ERK/MAPK activation in the dorsal hippocampus. *Sleep, 32*, 227–240.

Ribeiro, S., Goyal, V., Mello, C. V., & Pavlides, C. (1999). Brain gene expression during REM sleep depends on prior waking experience. *Learning and Memory, 6*, 500–508.

Ribeiro, S., Mello, C. V., Velho, T., Gardner, T. J., Jarvis, E. D., & Pavlides, C. (2002). Induction of hippocampal long-term potentiation during waking leads to increased extrahippocampal zif-268 expression during ensuing rapid-eye-movement sleep. *Journal of Neuroscience, 22*, 10914–10923.

Ribeiro, S., Shi, X., Engelhard, M., Zhou, Y., Zhang, H., Gervasoni, D., et al. (2007). Novel experience induces persistent sleep-dependent plasticity in the cortex but not in the hippocampus. *Frontiers in Neuroscience, 1*, 43–55.

Seibt, J., Dumoulin, M. C., Aton, S. J., Coleman, T., Watson, A., Naidoo, N., et al. (2012). Protein synthesis during sleep consolidates cortical plasticity in vivo. *Current Biology, 22*, 676–682.

Smith, C. (1995). Sleep states and memory processes. *Behavioural Brain Research, 69*, 137–145.

Smith, C. (1996). Sleep states, memory processes and synaptic plasticity. *Behavioural Brain Research, 78*, 49–56.

Tononi, G., & Cirelli, C. (2006). Sleep function and synaptic homeostasis. *Sleep Medicine Reviews, 10*, 49–62.

Tononi, G., & Cirelli, C. (2014). Sleep and the price of plasticity: From synaptic and cellular homeostasis to memory consolidation and integration. *Neuron, 81*, 12–34.

Veyrac, A., Besnard, A., Caboche, J., Davis, S., & Laroche, S. (2014). The transcription factor Zif268/Egr1, brain plasticity, and memory. *Progress in Molecular Biology and Translational Science, 122*, 89–129.

Vyazovskiy, V. V., Cirelli, C., Pfister-Genskow, M., Faraguna, U., & Tononi, G. (2008). Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. *Nature Neuroscience, 11*, 200–208.

Walker, M. P. (2009). The role of sleep in cognition and emotion. *Annals of the New York Academy of Sciences, 1156*, 168–197.

Wang, S. H., & Morris, R. G. (2010). Hippocampal-neocortical interactions in memory formation, consolidation, and reconsolidation. *Annual Review of Psychology, 61*(49–79), C41–44.

Whitlock, J. R., Heynen, A. J., Shuler, M. G., & Bear, M. F. (2006). Learning induces long-term potentiation in the hippocampus. *Science, 313*, 1093–1097.

Winson, J., & Abzug, C. (1977). Gating of neuronal transmission in the hippocampus: Efficacy of transmission varies with behavioral state. *Science, 196*, 1223–1225.

Yang, G., Lai, C. S., Cichon, J., Ma, L., Li, W., & Gan, W. B. (2014). Sleep promotes branch-specific formation of dendritic spines after learning. *Science, 344*, 1173–1178.

Please cite this article in press as: Ravassard, P., et al. Paradoxical sleep: A vigilance state to gate long-term brain plasticity? *Neurobiology of Learning and Memory* (2014), http://dx.doi.org/10.1016/j.nlm.2014.11.013